1852-Pos Board B762
Modulation of Membrane Curvature Sensitivity in Protein-Membrane Interactions by Bulk Lipid Composition
Benjamin R. Capraro, Tobias Baumgart.
Many cellular processes depend on the ability of certain proteins to localize differentially between membrane regions of different curvature. For proteins that exhibit this property, such as BAR domain proteins, the generality–with respect to lipid composition–of the sensitivity of their interaction with membranes to membrane curvature has not been adequately assessed. Here, we investigate the possibility that the bulk composition of the underlying bilayer may impact the curvature sensitivity of protein distribution on membranes. We use membrane tethers extracted from micropipette-aspirated giant unilamellar vesicles to study the partitioning of the Drosophila amphiphysin N-BAR domain between membrane regions of different curvature by confocal fluorescence microscopy. Our measurements strikingly reveal a difference in curvature partitioning depending on the bulk lipid composition of the membrane. We show that the differences in partitioning observed cannot be rationalized by differences in membrane binding affinity between the compositions investigated. Thus, in addition to structural features of proteins, properties of membrane lipids can act as determinants of curvature sensitivity in membrane-protein interactions.

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Lipid Lateral Organization on Giant Unilamellar Vesicles Containing Lipopolysaccharides
Jakub Kubiak, Jonathan Brewer, Søren Hansen, **Luis A. Bagatolli**.
We developed a new protocol to generate giant unilamellar vesicles (GUVs) composed of mixtures of a single lipopolysccharide (LPS) specie and E. coli polar lipid extracts at physiological salt and pH conditions. Four different LPSs differing in the size of the polar head group (i.e. LPS smooth > LPS-Ra > LPS-Rc > LPS-Rd) were selected to generate the GUVs. Quantitative analysis of LPS concentration in the final membranes, shows that LPS partition into the membrane saturates in a LPS-structure depending manner, i.e. not more than 15 mol % for LPS-smooth and LPS-Ra, and up to 25 mol % for LPS-Rc and LPS-Rd respect to total lipids is incorporated in the final membrane. These GUVs were used to evaluate the impact of different LPS species on the lateral structure of the host membrane (i.e. E. coli polar lipid extract). Rhodamine-DPPE labeled GUVs show the presence of elongated micrometer sized lipid domains for GUVs containing either LPS-Rc or LPS-Rd above 10 mol %. LAURDAN GP images confirm this finding and show that this particular lateral scenario corresponds to the coexistence of fluid disordered and gel (LPS enriched) -like micron sized domains, similar to that observed when LPS is replaced with lipid A. For those LPSs containing the more bulky polar head group (i.e. LPS-smooth and LPS-Ra), absence of micrometer sized domains is observed for all LPSs concentration explored in the GUVs. However, fluorescence correlation spectroscopy (using fluorescently labeled LPS) and LAURDAN GP experiments in these microscopically homogeneous membranes suggest the presence of LPS clusters with dimensions below our microscope's resolution (~300 nm). Our results indicate that LPSs show a tendency to cluster into laterally stiff (gel -like) domains and the size of these domains depends on LPS's chemical structure and concentration.

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Analysis of Interleaflet Domain Registry in Phase-Separated Lipid Bilayers
Gregory G. Putzel, Mark J. Uline, Igal Szleifer, Michael Schick.
We present a statistical mechanical model of fluctuating domain interfaces in a phase-separated lipid bilayer where the two leaflets are coupled. The model is solvable by means of its exact correspondence with a one-dimensional quantum mechanics problem. We exploit this correspondence to derive an expression for the characteristic separation between the interfaces in the two leaflets. For any reasonable value of the interleaflet coupling, the model predicts a characteristic separation below optical resolution, thus explaining the absence of visible fluctuations out of interleaflet registry of domains in phase-separated model membranes.
In addition we have calculated the interleaflet coupling in liquid-liquid phase-separated lipid bilayers using a molecular mean field model. We obtain composition-dependent values of order 0.01 kT per square nanometer, an order of magnitude smaller than previous estimates.

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Theory of Domain Formation on Model Myelin Monolayer System
Dong Woog Lee, Younjin Min, Joseph A. Zasadzinski, Jacob N. Israelachvili.
Lipid domains indicate the coexistence of thermodynamic phases of lipid molecules at hydrophobic-hydrophilic interface. Domain size, shape and distribution are directly affected by the line tension (λ) and dipole density difference (m) at domain boundaries [1-2]. In this work, a thermodynamic equation expressing domain size distribution was derived and fitted to domain distributions from fluorescence images of both healthy (control) and experimental allergic encephalomyelitis (EAE) cytoplasmic (CYT) model myelin monolayers. From these fits we simultaneously extract λ (in units of fN) and m (in units of pC/m^2). Both parameters λ and m decrease with increasing surface pressure (Π). Moreover, the control monolayer had higher values of λ and m compare with EAE monolayer for all values of Π where domains can be observed clearly. Based on the difference of these values, EAE monolayer seems to be more 'stressed' than control monolayer. This indicates an important relationship between Π, domain size and distribution, λ and m, which can be used to point out the potential cause of demyelinating diseases like Multiple Sclerosis (MS) at molecular scale.

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Detecting Critical Fluctuations in Ternary Lipid and Cholesterol Mixtures
Miranda L. Schmidt, James H. Davis.
Mixtures of long chain saturated and unsaturated phospholipids with cholesterol have attracted a lot of attention because of the formation of two coexisting fluid bilayer phases over a broad range of temperatures and compositions of these systems. Davis *et al.* and Veatch *et al.* have presented phase diagrams for ternary mixtures of two phospholipids (DOPC and DPPC-d_{62}) and cholesterol. These ternary mixtures show evidence of critical fluctuations in composition [1,2]. A preliminary investigation of the linewidth of the deuterium NMR spectrum of powder samples under magic angle spinning (MAS) has been done and shows a large increase (5 or 6 fold) in the spectral linewidths as we approach the critical temperature. We also looked at the MAS spectral linewidths for these samples using proton NMR but found that the effect is very minimal and inconclusive. We are undertaking to explore these systems in more detail using static solid-state NMR techniques. We are using a Jeener Echo pulse sequence which probes the relaxation of the nuclear magnetic dipolar spin energy to the lattice. The Jeener Echo pulse sequence makes dipolar order, which ordinarily is much smaller than the Zeeman order, detectable by NMR [3]. Dipolar order relaxes by fluctuations in the local fields of the nuclei which are typically a few kHz in strength. Critical fluctuations would cause changes in the local fields and in turn changes in the rate of relaxation of dipolar order. This technique is sensitive to slow motions which makes it useful for investigating the existence of fluctuations near a critical point.
[1] Davis, J.H. *et al.* (2009) Biophys. J. 96:521-539. [2] Veatch, S.L. *et al.* (2007) Proc. Natl. Acad. Sci. USA 104:17650-17655. [3] Jeener, J. and Broekaert, P. (1966)Phys. Rev. 157:232-240.